2.95 ± 0.082; component parallel with fiber axis: 1.05 ± 0.015 vs. 1.90 ± 0.057; component perpendicular to fiber axis: 1.21 ± 0.014 vs. 1.85 ± 0.062). Similar differences in amplitude and FWHM values were observed on line-scan images. On these images spark pairs close enough to be detected as single events were more abundant compared to simulated images containing the same number of randomly distributed sparks. Calcium sparks had significantly slower rising phase under both conditions as compared to the control. Complex events corresponding to overlapping sparks with differently located centers of signal mass on consecutive x-y frames were also found on line-scan images.

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Quantification of the CICR Response to Artificial Ca Sparks in Striated Muscle

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In cardiac muscle physiologic Ca^{2+} release is CICR, but in skeletal muscle the role of CICR remains unsettled. ''Artificial Ca^{2+} sparks'' of up to 8 μM were produced by 2-photon irradiation of Ca^{2+} cages, applied in the near vicinity of membrane-permeabilized skeletal fibers from frogs and mice, and rabbit atrial and ventricular myocytes, while confocally imaging their Ca^{2+} response with Fluo-4FF. In response to transients as low as 0.28 μM, frog fibers produced waves (n=16) that satisfied the definition of CICR (Endo, PhRev 2009), including inhibition by Mg^{2+}. Cardiomyocytes responded similarly, with waves that could propagate fully or partially through the cells. Responses were absent in mouse skeletal fibers (n=51) unless RyR opening was promoted pharmacologically (n=24). The underlying Ca^{2+} flux was calculated from fluorescence images by generalizing the ''backward'' method (Ríos et al, JGP 1999). In frog fibers, fluxes were found to reach 55 mM/s, which is close to values measured under voltage clamp depolarization. A positive three-way correlation was found among flux, speed and extent of propagation. Accordingly, the responses of cardiomyocytes and pharmacologically conditioned mammalian fibers, both of which had smaller flux, were of lower velocity and sometimes limited propagation range. The quantitative properties of the propagating wave, in all cases, were well described by a simple continuum model of sources and sinks, with a common threshold $[Ca^{2+}]$ for channel opening (Kupferman et al, BJ 1997). This description suggests a fundamental commonality of mechanisms. The lower flux and higher threshold $[Ca^{2+}]$ in mice are consistent with the notion that CICR is supported by isoform 3 or beta (in the frog), and that isoform 1 *in situ* is less susceptible to CICR. Supported by NIAMS and NHLBI (NIH).

Calcium Signaling Pathways

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Excitation Coupled Calcium Entry (ECCE) is Enhanced in mdx Myofibers and is Modulated by ROS

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Altered Ca^{2+} homeostasis and elevated ROS have been implicated in the pathogenesis of Duchene Muscular Dystrophy (DMD). In these studies we investigated the contribution of Excitation Coupled Calcium Entry (ECCE) to the altered Ca^{2+} signaling phenotype and whether this Ca^{2+} influx pathway is redox sensitive. Adult, post necrotic *mdx* mice (e.g., > 4 months) exhibited significantly enhanced basal Ca^{2+} influx at rest compared to WT as determined by Mn^{2+} quench. Upon depolarization, Ca^{2+} influx (i.e., ECCE) was significantly enhanced in the *mdx* compared to WT. Scavenging ROS with n-acetylcysteine (NAC) did not alter the basal Ca^{2+} influx in the WT or *mdx* muscle fibers. NAC, however, significantly reduced ECCE in *mdx* muscle by approximately 66% with no effect on WT. The non-specific TRP channel inhibitor 4-methy-4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide (BTP-2) reduced basal Ca^{2+} influx and ECCE in *mdx* muscle fibers by 51% and 72% respectively. In WT, BTP2 reduced ECCE by 41% and had no effect on basal Ca^{2+} permeability. We conclude that basal Ca^{2+} influx and ECCE are likely TRP dependent and only ECCE in the *mdx* can be modulated by ROS scavenging. Funding: LPM - 5F31NR011245-03, CWW- RC2 NR011968, GGR-K01AR051519

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Effect of Oxidative Stress on Intracellular Ca^{2+} and Na^{+} Concentration in Duchenne Muscular Dystrophy Myotubes

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Duchenne muscular dystrophy (DMD) is an X-linked myopathy leading to progressive muscle weakness that is the most common and devastating type of muscular dystrophy in boys. Oxidative stress has been suggested as a potential upstream contributor to the skeletal muscle damage in DMD. Here we test this hypothesis by comparing the effect of ROS in intracellular concentrations of Ca^{2+} and Na^{+}. Intracellular calcium concentration ($[Ca^{2+}]_i$) and intracellular Na^{+} concentration ($[Na^{+}]_i$) were measured using Ca^{2+} and Na^{+} selective microelectrodes in Wt and mdx myotubes. Mdx myotubes showed significantly elevated $[Ca^{2+}]_i$ and $[Na^{+}]_i$ in comparison to Wt cells. In mdx myotubes $[Ca^{2+}]_i$ was 333 ± 35 nM (mean ± SD, n=35) and $[Na^{+}]_i$ was 17.5 ± 1.3 mM (n=15) *versus* 115 ± 10 nM (n=40) and 7.9 ± 0.6 mM, (n=15) in Wt. Ca^{2+} influx at rest measured by Mn^{2+} quenching also reveled increased Ca^{2+} entry in mdx. The effect of ROS on cultured myotubes was tested by exposing myotubes to 10μM H_2O_2, a concentration that did not alter cell viability. H_2O_2 caused a significant elevation of $[Ca^{2+}]_i$ and $[Na^{+}]_i$ in both mdx and Wt. However, the relative increase of both ions was much greater in mdx than Wt. Whereas in mdx $[Ca^{2+}]_i$ reached 1167 ± 138 nM (n=14) and $[Na^{+}]_i$ 23.6 ± 2 mM (n=11) in Wt cells these concentration were 152 ± 11 nM (n=15) and 10.5 ± 0.5 mM (n=14), respectively. In addition, pre-treatment of mdx and Wt myotubes with nonselective TRPC blockers 5μM BTP2 or 20μM gadolinium prevented the increase in $[Ca^{2+}]_i$ and $[Na^{+}]_i$ induced by H_2O_2. These results suggest that mdx myotubes are more susceptible than Wt to elevation of $[Ca^{2+}]_i$ and $[Na^{+}]_I$ induced by oxidative stress. Moreover, H_2O_2-induced dysregulation of $[Ca^{2+}]_i$ and $[Na^{+}]_i$ seems primarily mediated by Ca^{2+} entry.
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Intracellular Sodium Overload Contributes to Deterioration of Function in Dystrophic Heart

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$[Na^{+}]_i$ is found to be elevated in several models of heart failure. This is believed to favor Ca^{2+} extrusion from mitochondria via the mNCX and therefore reduce mitochondrial Ca^{2+} accumulation. As several dehydrogenases of the mitochondrial Krebs cycle are stimulated by Ca^{2+}, the defects in cellular sodium homeostasis may induce mitochondrial oxidative stress. Previously we have estimated that resting $[Na^{+}]_i$ is significantly elevated in ventricular myocytes isolated from dystrophic (*mdx*) mice compared to those from wild-type (WT) mice (24.2 ± 3.1 mM, n=13 *vs.* 14.0 ± 1.7 mM, n=9). Moreover, the mitochondrial matrix was significantly oxidized at resting conditions (35 ± 3.1 %, n=27 *vs.* 53 ± 5.2 %, of NADH reduction n=10, in *mdx* and WT respectively). Here maximal oxidation of NADH by FCCP/oligomycin was taken as 0% reduced NADH and maximal reduction by rotenone and β-hydroxy-butyrate was defined as 100% reduced NADH. We suggested that beat-to-beat mechanical activity in dystrophic heart might lead to greater $[Na^{+}]_i$ accumulation and oxidation of the mitochondrial matrix. Here we investigated whether elevated $[Na^{+}]_i$ affects mitochondrial energetics during excitation-contraction coupling in dystrophic myocytes. We measured NADH autofluorescence in WT and *mdx* cells under control conditions and during 4Hz stimulation in the presence of isoproterenol. An abrupt increase in workload resulted in significant oxidation of NADH both in *mdx* and WT cardiomyocytes. However, the extent of oxidation was significantly greater in dystrophic cells (50 ± 9 %, n=10 *vs* 15 ± 6 %, n=11 decrease in NADH, respectively). When workload was discontinued, the mitochondrial matrix quickly returned back to its base line levels in WT but not in *mdx* cells. These results support the notion that the elevated $[Na^{+}]_i$ contributes to the impaired energetics in dystrophic cardiomyocytes and therefore contributes to the development of dystrophic cardiomyopathy.